# Determination of Spatial and Temporal Trends of *Enterococcus* Concentrations Measured at Two Environmental Sampling Areas in Ocean and Monmouth County New Jersey Using Real Time PCR and Membrane Filtration

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## 1.0 PROJECT SUMMARY

Cell densities of the fecal pollution indicator genus, *Enterococcus*, were determined by a rapid (4 h or less) quantitative polymerase chain reaction (qPCR) analysis method in 50 mL water samples collected from two environmental marine sampling areas in New Jersey during the summer of 2008. Measurements by qPCR were compared to counts of *Enterococcus* colony-forming units (CFU) determined by Method 1600, membrane filter (MF) analysis using mEI agar.

Samples from stations in Monmouth County, Myron/Wilson Bay, Neptune City (renamed Memorial Park) and Ocean County, Central Avenue, Island Heights, NJ were collected over an 8 week period in July and August, 2008. These sites were chosen because they were represented by the highest bacterial contamination based on a study by Ferretti, et al, 2008 and historical data from 2005-2007 from the Cooperative Coastal Monitoring Program database from NJDEP. Geometric means for *Enterococcus* were 2 times lower at Myron/Wilson and 15 times lower at Central Avenue in 2008 as compared to data collected at the same stations in a prior study conducted in 2007 (Ferretti, et. al. 2008). There was only one excursion of the 104 CFU/100 mL water quality criterion for *Enterococcus* at Central Avenue and two at Myron/Wilson Bay over the entire 8 week study period.

At all sampling sites, the geometric means of *Enterococcus* concentrations in water samples exhibited lognormal distributions over the study period for both qPCR and MF. The study design focused on the spatial and temporal variability of qPCR and MF method results. To determine spatial variability, samples were collected along an 80 M transect at three equidistance locations across the transect at Myron/Wilson Ave and Central Avenue and split for analysis by the two methods. Spatial variability was low for both qPCR and the MF method.

Samples were collected each week over an 8 week period at both sites to determine temporal variability. Temporal variability was further evaluated by sampling three times

over a 24 hour period. Samples were collected between 6:00 and 9:00 am in the morning, then a second sampling event occurred approximately 6 hours later, and finally, a third sample was collected the following morning. This experimental design component was used to evaluate changes in *Enterococcus* concentrations determined by qPCR and MF over a 24 hour period and incorporate variability assessments based on changes in tidal cycle. Differences were seen in *Enterococcus* concentrations based on time sampled. On two of these occasions, concentration determined by qPCR and MF exhibited similar changes. On four other occasions, changes in MF and qPCR results did not agree over the 24 hour period. However, most of these differences were observed when *Enterococcus* concentrations were low (<104 cfu/100 mL). Overall *Enterococcus* concentrations were too low to examine the influences of other variables including tidal cycle, wind direction, and time of day on qPCR and MF results.

The geometric means ranged from 2.6 to 2096 calibrator cell equivalents (CCE) by qPCR analysis and 5.0 to 1805 CFU by MF analysis at Myron/Wilson Bay, Monmouth County (*N*=160). The geometric means from the samples collected at Central Avenue in Ocean County were 4.3 to 232 CCE/100mL by qPCR and 5.0 to 266 CFU/100 mL by MF (*N*=180). Within and between station (sample location) variability calculations were similar for MF and qPCR from this study. The within and between station variability from this study was slightly higher at these 2 sampling stations as compared to 2007 data from the same sampling stations (Ferretti et al 2008). Regression analysis of these results showed a significant positive correlation between qPCR and MF methods with an overall correlation coefficient (*r*) of 0.74. This relationship was similar to the one calculated for the 2007 evaluation of 20 beach/bay samples in Ocean and Monmouth Counties (r = 0.71) (Ferretti et al 2008).

The endpoints for qPCR and MF are not directly comparable. Estimates by qPCR are expressed as Calibrator Cell Equivalents, which is a mathematical computation based on comparison of gene sequences recovered from the test samples with those from spikes of known numbers of *Enterococcus* cells in similarly-processed and analyzed calibrator samples. However, the results presented in this report provide a comparison of relative trends in indicator densities determined by the different methods. MPN and CFU per mL

are the benchmark endpoints and comparison to CCE is provided as a way to express the relative changes between qPCR and the conventional methods.

Before qPCR can be a stand alone technology for beach management decisions, additional data regarding intra- and inter-laboratory variability, especially use of different qPCR platforms and reagents, must be evaluated. Also, there is a need to evaluate epidemiological data in conjunction with qPCR data to help formulate appropriate risk values. Epidemiological studies are being performed by USEPA as part of the National Epidemiological and Environmental Assessment of Recreation Water program (NEEAR) study using qPCR data and Method 1600 MF procedures. Once completed, then site specific factors affecting qPCR results should be evaluated for any target sampling area.

## 2.0 INTRODUCTION

There is a need for more rapid methods for the determination of microbial water quality at bathing beaches. It has been demonstrated that densities of bacteria from the genus *Enterococcus* in both marine and freshwater samples are directly correlated with gastroenteritis illness rates in exposed swimmers (Cabelli 1982, Dufour 1984, Wade 2006). USEPA requires that recreational waters across the United States be monitored routinely for *Enterococcus* spp. and /or *Escherichia coli*. While neither of these organisms is pathogenic, both are considered to be surrogates for the presence of bacterial and viral pathogens found in fecal material. Currently, approved methods for measuring concentrations of *Enterococcus* and *E. coli* in recreational waters include MF (MF), Most Probable Number (MPN) techniques and Defined Substrate Technology (DST®) tests. Although these methods have been refined over the years, results are not available for 24 hours. Due to the fluctuating nature of microbial contamination, this delay makes it difficult for beach managers to make decisions regarding beach closures and/or swimming restrictions. At best, decisions are made using one day old information; or a decision regarding safe beach usage is not made until results of a confirmation test are available, which may be up to 72 hours after the initial “failed” test was sampled. Because microbial water quality can change rapidly (Boehm et. al., 2002), guidelines based on indicator organisms that require 18-24 hours to develop, may result in both

unnecessary beach closings or exposure of swimmers to poor microbial water quality. A study by Kim and Grant (2004) estimates that up to 40% of beach closures may be in error.

The use of qPCR assays has shown promise as an alternative technology for monitoring microbial water quality at recreational beaches (Haugland, et. al. 2005; Wade, et. al, 2006, Ferretti, et al, 2008). Primer sets and probes are available for the specific detection of *Enterococcus* as well as other fecal indicator and pathogenic microorganisms using real time or quantitative PCR (qPCR) (Ludwig and Schleifer (2000), Lyon (2001), Brinkman et al. (2003), Foulds et al. (2002), Blackstone et al. (2003), Frahm and Obst (2003), Guy et al. (2003), Noble et al. (2003). Protocols for qPCR are now available for quantifying indicator bacteria in recreational waters in approximately 3-4 hours. Because these methods provide a more rapid assessment of water quality, they have the potential to improve the timeliness of decision making for those responsible for beach management decisions. A positive correlation was observed between *Enterococcus* qPCR and the MF results at two freshwater beaches in a 10 week study conducted by Haugland et al., 2005.

In 2007, USEPA Region 2, New Jersey Department of Environmental Protection (NJDEP), and Ocean and Monmouth County Health Departments collaborated on a comparison study using qPCR and conventional microbiology methods at 20 beaches in Ocean and Monmouth Counties. Ocean and bay samples with varying levels (based on historical data) of expected microbial densities were sampled 10 times between June 18 and August 20, 2007. Cell densities of Enterococcus, were determined by qPCR, as well as two conventional 24 hour test methods (MF and Enterolert ®). Over 1000 samples were analyzed. In general, when *Enterococcus* concentrations were low using MF, qPCR results followed the same trend. qPCR concentrations increased as MF results increased. Regression analysis of these results showed a significant positive correlation between qPCR and MF methods with an overall correlation coefficient of 0.71. Additional data are needed to further refine qPCR technology for routine use at marine bathing beaches, including an inter-laboratory method validation study and an epidemiological study using qPCR water quality data. Close to 70 percent of the samples analyzed in 2007 revealed

low to non-detectable (ND) quantities of *Enterococcus*. qPCR compared favorably with the conventional methods at the low end of the *Enterococcus* measurements.

The information presented in this paper is a follow up to the project and was designed to further evaluate the performance of qPCR with an emphasis on spatial and temporal variability. One environmental sampling station from Monmouth County, Memorial Park (formally known as Myron/Wilson Bay, Neptune City, New Jersey) and one station from Ocean County (Central Avenue, Island Heights, New Jersey) were established for this study. Samples were collected along an 80 M transect and resampled at the mid-station of each transect 6 hours later and then 24 hours from the original weekly sampling event in each county. Samples were collected weekly at each station for a total of eight sampling events at each location. An additional sampling event following a rain event of at least 0.25" rain was performed at Memorial Park. The relationship of qPCR and MF methods were compared based on collection of samples at varying tidal cycles (sample in the morning and then sample the opposite tide in the afternoon). Also, a 24 hour resample was performed to evaluate changes in *Enterococcus* between the methods.

The purpose of this study was to compare *Enterococcus* concentrations using qPCR technology to the conventional MF testing method at two marine environmental sampling areas to evaluate temporal, spatial, and physical chemical observations/measurements and their relationship to *Enterococcus* concentrations measured using qPCR and MF.

## 3.0 METHODS AND MATERIALS

### 3.1 Study Sites

One environmental sampling station from Monmouth County (Memorial Park, formally known as Myron/Wilson, Neptune City, New Jersey) and Ocean County (Central Avenue, Island Heights, New Jersey) were established for this study (Figure 1). Four replicate samples were collected weekly at three locations across an 80 M transect, then resampled at the mid station of the bracket after 6 hours (PM event), and then 24 hours


Figure 1. Location map of sampling areas from the qPCR versus Membrane Filtration Study, July-August 2008.

later. Sampling was performed weekly between July 10, 2008 and August 27, 2008. Sampling sites were selected based on historical microbiological monitoring data and *Enterococcus* results from the 2007 study. Both study areas are environmental sampling areas and historically have exhibited overall higher concentrations of *Enterococcus* as compared to the existing sampling sites in the NJDEP CCMP universe.

**3.2 Water Sampling**

Samples were collected following procedures outlined by NJDEP in the Cooperative Coastal Monitoring Program (CCMP), Quality Assurance Project Plan, FY07/FY08, and Section 12.1, Sample Collection; in Chapter IX (Public Recreational bathing) of the State Sanitary Code, N.J.A.C. 8:26-1 et seq. (amended April 2004) and described briefly here. Samples were collected in sterile HDPE containers in an area with a stabilized water depth between the sampler's lower thighs and chest. The sample container (500 mL sterilized HDPE wide mouth jars, Nalgene® or equivalent) was placed approximately 8-12 inches below the water surface with the lid and stopper still attached. With the collector's arms extended to the front, the container was held near its base and downward at a 45-degree angle. The cap was removed and the container filled in one slow sweeping motion. The mouth of the container was kept ahead of the collector's hand and the container recapped while it is was still submerged. The cap remained submerged during sample collection. Sample remaining from microbiological analysis was used for turbidity and salinity analyses. A total of four independent (true) replicate samples were collected at each station for MF (EPA Method 1600) and qPCR filtration and analysis (USEPA Region 2 SOP BIO-10.1).

A 40 m transect, parallel to the shoreline, at a depth between the sampler's lower thigh and chest was established and sampled at three locations (4 replicates each), equidistance along the transect. The midpoint of this transect was sampled approximately 6 hours later (4 replicates) and also 24 hours from the initial transect sampling. The mid point of the transect established for both sampling areas were sampled on separate events within 24 hours of a rain event which produced 0.25 inches or more of rain.

All AM samples were collected between 6:00 and 9:00 am and afternoon collections were performed between 1:00 pm and 3:00 pm. Four replicate samples were collected in each afternoon and 24 hour sampling event at the mid station of the transect established at both stations.

Time and date of sample collection, tide stage, air and water temperature, rainfall, wind direction, and other general conditions were documented and recorded. Following collection, all samples were placed in coolers with ice during transport to the laboratory and stored at 1–5 °C prior to filtration in the laboratory. Sample filtration was completed within 6 h of collection. The filters for the qPCR analysis were frozen immediately at -20 to -70$^{o}$C until analysis. Turbidity at the mid-station of each AM sampling event was measured using Standard Methods, American Public Health Association (20$^{th}$ Edition). Salinity was measured via conductance bridge (YSI, Model 85) or refractometer.

**3.3 Microbiological Procedures, Method 1600, Membrane Filtration (MF)**

*Enterococcus* was enumerated by EPA Method 1600 on mEI agar plates (US EPA 2006). Volumes of 10 mL from each water sample were filtered on 47-mm diameter, 0.45 μm pore size, membrane filters (Millipore Corp., Billerica, MA or equivalent). The filters were incubated on plates of mEI agar for 24 hours at 41±0.5 °C before determining colony numbers. *Enterococcus* by MF was expressed as CFUs per 100 mL of water. Monthly verification tests of 10 typical and 10 atypical were performed for each batch of water samples collected over the study period. Each preparation of mEI agar was tested for performance (i.e., correct enzyme reaction) using pure cultures of target and non-target organisms. Sterility of the filters and phosphate-buffered water used for rinsing the filtration apparatus was also tested with each batch of samples received by the laboratory. BioBalls™, TCS Biosciences, LTD, which contain a certified number of bacterium, were used routinely for determination of Ongoing Precision and Recovery.

## 3.4 qPCR Procedures

DNA extraction, amplification and detection of *Enterococcus* were based on previously reported protocols, Brinkman (2002) and Haugland (2005).

### *3.4.1 Test Sample Filtration Procedure*

Fifty mLs of each test sample were filtered through a 0.4 micron, 47 mm diameter polycarbonate filter fitted in a pre-sterilized disposable 250 mL filter funnel within 6 hours of collection. The filter paper was folded in half and folded longitudinally 2-3 more times before being placed into a 2.0 mL polycarbonate preloaded bead tube (Gene-Rite S0201-50) using sterile forceps. The tubes with the polycarbonate filter paper were frozen at -20 to -70 $^{o}$C until ready for use in the qPCR analysis.

### *3.4.2 Test Filter Sample Extraction Procedure*

Salmon testes DNA extraction buffer was prepared in advance of the DNA extraction procedure. Salmon testes DNA extraction buffer acts as an exogenous, positive control and reference. Initially, the concentrated salmon testes DNA (Sigma, D1626, and St. Louis, MO) was re-suspended in water and was diluted with AE buffer (Qiagen, Cat No. 19077, Valencia, CA) to obtain the target concentration required for the procedure. 590 uL of a 0.2 ug/mL of salmon testes DNA extraction buffer mix was added to 2.0 mL tubes containing silica beads (GeneRite, #S0205-50, North Brunswick, NJ) and the negative control filter blank or test sample filter. The extraction tubes were subjected to bead beating in an eight position mini bead beater (Biospec Corp., Bartlesville, OK) for 1 minute at a rate of 5,000 rpm and were then centrifuged at 12,000 x g for 1 minute to pellet the glass beads and debris. The DNA in the supernatants from the extraction tubes was transferred to sterile 1.7 mL microcentrifuge tubes and then centrifuged for additional 5 minutes at 12,000 x g to further remove any sediments. The final

genomic supernatant was either analyzed immediately or stored at -20 °C until analysis by qPCR.

*3.4.3 Enterococcus faecalis culture procedure*

A pure culture of *E. faecalis*, ATCC 29212, was inoculated in a 20 mL test tube with 10 mL of brain heart infusion broth (BHI, Difco, #Ref 237500, Sparks, MD). The culture was incubated on a shaker for 24 ± 2 hours at 35 °C ± 0.5 °C. Also, a non inoculated tube was placed in the incubator to test the sterility of BHI broth. The cell culture was transferred to 15 mL conical tubes and centrifuged at 6000 x g for 5 minutes to pellet the cells. The supernatant was discarded, and the cell pellet was washed twice with 10 mL of a 1x phosphate-buffered saline (PBS) (Invitrogen, Cat. No. 14190, Carlsbad, CA) and resuspended in 5 mL of 1x PBS solution. The optical density of the *E. faecalis* cell suspension was quantified on a NanoDrop spectrophotometer ND-1000 v3.3.1 (Wilmington, DE). The *E. faecalis* cell suspension was divided into 6 microcentrifuge tubes, each one containing 500 uL for preparation of purified genomic DNA standards. The remaining cell suspension was dispensed by 10 uL aliquots into 100-200 microcentrifuge tubes, which were used to prepare calibrator samples for subsequent qPCR analyses.

*3.4.4 Calibrator and DNA extraction and preparation procedure*

A 10 uL aliquot of *E. faecalis* cell suspension was spotted onto a blank polycarbonate filter which was then transferred to an extraction tube containing pre-loaded glass beads and 590 uL of 0.2 ug/mL salmon testes DNA extraction buffer as described in section 3.4.2. The tube was shaken by a mini-bead beater for 1 minute at 5,000 rpm and then centrifuged at 12,000 x g for 1 minute to pellet the glass beads and debris. The genomic DNA in the supernatants from the extraction tubes was transferred to sterile 1.7 mL microcentrifuge tubes and then centrifuged for additional 5 minutes at 12,000 x g. The final DNA-containing supernatant was either analyzed immediately or stored at -20 °C until analysis.

The *E. faecalis* cell suspensions used to prepare the calibrator samples were also used to create purified genomic DNA standards for *E. faecalis*. Two 500 uL undiluted *E. faecalis* cell suspensions were placed into a 2.0 mL preloaded tube containing glass beads, extracted in the mini-bead beater, and centrifuged. The supernatant was transferred to another tube. The genomic DNA supernatant was then digested with 1 uL of 5 ug/uL RNase A (Sigma, R-4642, St. Louis, MO) for 1 hour at 35 °C. The RNase A was used to digest the RNA in the sample to facilitate purification of the genomic DNA of *E. faecalis*. After RNase digestion, the DNA was purified by DNA-EZ purification kit (GeneRite, K102-02C-50, North Brunswick, NJ). The concentration of DNA was then measured on the NanoDrop spectrophotometer. The DNA was considered to be acceptable if the $OD_{260}/OD_{280}$ reading was $\geq 1.75$.

*3.4.5 qPCR assay preparation and detection procedure*

Each reaction tube contained assay mix with a total volume of 25 uL. The qPCR assay mix had the following components: 12.5 uL of TaqMan Universal Master Mix (Applied Biosystems, Part Number 4304437); 2x concentrated; 1 uL of forward primer (1 μM); 1 uL reverse primer (1 μM); 1.5 uL of a fluorogenic probe (0.08 μM); 2.5 uL of 2 mg/mL ultra pure bovine serum albumin (Ambion, Cat # AM2616); 1.5 uL of sterile water and 5 uL of diluted DNA template (5 fold dilution). TaqMan Universal Master Mix consisted of AmpliTaqGold DNA polymerase, AmpErase UNG, dNTPs with UTP, passive reference 1 and optimized buffer components. The published primer sequences were ECST748F: 5'-AGAAATTCCAAACGAACTTG, ENC854R: 5'-CAGTGCTCTACCTCCATCATT and GPL813TQ: 5'-6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA was used for the probe (Ludwig and Schleifer, 2000). These sequences are homologous to the large subunit ribosomal RNA genes of all reported species within the *Enterococcus* genera. Published primers and hybridization probe sequences for salmon DNA assay were SketaF2: 50-GGTTTCCGCAGCTGGG for the forward primer; SketaR3: 50-CCGAGCCGTCCTGGTCTA for the reverse primer; and SketaP2:

50-6FAM-AGTCGCAGGCGGCCACCGT- TAMRA for the probe. These sequences are homologous to internal transcribed spacer region 2 of the ribosomal RNA gene operon of chum salmon, *Oncorhynchus keta*, Domanico et al. (1997). Primers and fluorescently labeled probes were purchased from Applied Biosystems Inc. (Foster City, CA).

Each reaction tube containing 25 uL of the assay mix was then placed in a Smart Cycler II (Cepheid, Sunnyvale CA) for 45 cycles under the following conditions: 2 min at 50 °C, 10 min at 95 °C, 15 s at 95 °C and 2 min at 60 °C. Cycle threshold ($C_T$) values were calculated by the instrument. Cycle threshold values occurred when the amplification fluorescence growth curves crossed a threshold of 8 units that was established for this qPCR method. $C_T$ values for each sample were collected at the end of each run and saved in Excel format. A No Template Control (NTC), which tests the assay mix for contamination, was included with each batch of samples analyzed along with one field blank. Any positive amplification for the NTC and filter blank samples was reanalyzed for verification. A sample was considered below the limit of detection when the fluorescence threshold was not reached within 45 cycles.

### 3.5 qPCR Quality Control

Maintaining a contamination free process and environment is an important component of qPCR analysis. Decontamination of workstations, pipettes and equipment after each use was performed using a 70% ethanol solution and/or bleach solution. Pre-sterilized pipet tips manufactured with aerosol resistant filters were used in the steps of the DNA testing process. An ultraviolet workstation was used to maintain sterility of tips and reagents. Pre-sterilized disposable filters and housings were used during the test sample filtration process to prevent cross-over contamination. All reagents and supplies were tested and certified by the manufacturer for specificity, sensitivity and to be free of contamination. The primers and probes were dispensed in small aliquots to avoid contamination and degradation.

Contamination or misleading qPCR results can be detected by using positive and negative quality control samples, which were implemented throughout this study. A negative control (method blank), was performed in the lab to test for proper filtering technique and reagent sterility. A method blank was analyzed using DNase/RNase free sterile water as a sample, which was processed in parallel with the water samples. Another negative control was a No Template Control (NTC) which was used in each batch of samples tested by qPCR to verify the purity of the master mix, reagents, and ensure no contamination occurred during the processing of the test samples (the NTC consisted of DNase/RNase free sterile water or buffer).

The calibrator samples served as the positive control to validate that the master mix and reagent preparation by properly producing amplification of the target nucleic acid. Salmon DNA, added to each sample, served as the sample processing control (SPC) to indicate that there were no significant losses of DNA during sample extraction or PCR interferences from the test samples.

### 3.6 qPCR Data Analysis

The amplification efficiency of the *Enterococcus* qPCR assay was determined as the first step in the qPCR data analysis process. Amplification efficiency is defined as the rate at which a PCR amplicon is generated, normally doubling during each cycle (Applied Biosystem, 2004). The amplification efficiency is normally equal to 2, however, the reagents, assay preparation, purity of the samples and the inherent features of the primers, probe and target sequence can alter the efficiency to less than 2. Initially, purified and quantified *E. faecalis* genomic DNA was serial diluted to estimated concentrations 4 x $10^4$, 4 x $10^3$, 4 x $10^2$, 2 x $10^2$, and 1 x $10^2$ lsrRNA gene sequences per 5 uL. These standards were analyzed by qPCR in triplicate. $C_T$ values were obtained, averaged and subjected to regression analysis against the log 10-transformed target sequence per reaction in order to obtain the equation of the line for the standard curve. The DNA standard curve for this study was y = -3.33x + 35.32, where -3.33 is the slope. The slope value from the standard curve was used to calculate the amplification efficiency using the following formula AF = $10 \wedge ^{(1/ (-) \text{ slope value})}$. The calculated AF was 1.998. The $r^2$ value

from the DNA standard curve used during this study was 0.9998 . This amplification efficiency value was used in conjunction with the comparative cycle threshold method to estimate the target cell densities in the water filtrate extracts as calibrator cell equivalents (CCE) as previously described (Applied Biosystem (2004), Haugland et al (1999), Haugland et al (2005)). Target cell estimates in each test sample were then multiplied by 2 to express results as CCE per 100 mL sample volume.

Five-fold dilutions of the test sample filters and calibration extracts were analyzed in this study to minimize potential interferences from undiluted extracts of the saltwater samples. The $C_T$ values for salmon DNA assay in water filter samples with higher than 3 $C_T$ units above the mean values from the calibration extracts were reanalyzed. If reanalysis did not fall within expected results, the data were qualified.

### 3.7 Statistical Analysis

Arithmetic and geometric means were calculated on all microbiological results collected during the study. A $Log_{10}$ transformation was performed on all raw data. Standard deviation between and within sampling visits was determined on the Log10 transformed data. Coefficient of Variation (C.V.) calculations of within sampling visit raw geometric means were performed on data from each sampling station. A linear regression was calculated using the geometric means of MF versus qPCR results.

Differences in *Enterococcus* concentrations across a sampling transect and the PM and 24 hour samples were tested for assumptions of Normality and Variance using untransformed data. Significant differences of *Enterococcus* concentrations across transects, 6 Hour and samples collected 24 hours later were determined using either Tukey's Method of Multiple Comparisons (p=0.05, critical value = 4.49, parametric) or Kruskal Walis/Dunnetts Multiple Comparison Test, (p=0.05; crit value = 2.936, non-parametric) (ToxStat, University of Wyoming and Statistix 8, Analytical Software, Tallahassee, FL).

## 4.0 RESULTS AND DISCUSSION

Estimates by qPCR are expressed as CCE, which is a mathematical computation based on comparison of gene sequences recovered from the test samples with those from spikes of known numbers of *Enterococcus* cells in similarly-processed and analyzed calibrator samples. The unit of CFU per 100 mL is the conventional endpoint for MF. Comparison of CFU/100 mL to CCE /100 mL is provided in this study as a way to express the relative changes between qPCR and the conventional methods. Samples with no detectable bacterium were assigned values of 5 CFU per 100 mLs (equivalent to one-half the reporting limit of the MF method for a 10x dilution sample); and those with no detectable CCE's were assigned 2.5 CCE / 100 mL for qPCR analysis (equivalent to one-half the reporting limit of the qPCR method for a 5X diluted sample).

### 4.1 Summary Statistics

Sampling stations were selected because historically, they have exhibited long term trends of higher *Enterococcus* concentrations as compared to other stations that have been routinely monitored in each County. Both of the sampling stations including this study were sampled in 2007 as part of the initial qPCR versus MF/Enterolert study. The 2007 data will serve as a comparative dataset for the 2008 sampling effort.

The arithmetic mean of qPCR and MF results were similar at the Central Avenue site between 2007 and 2008. *Enterococcus* via qPCR was higher in 2008 than 2007 at Myron/Wilson Bay. This increase was due to a spike in *Enterococcus* following a rain event on July 30, 2009 (Table 1a). The Log10 standard deviation (SD) within sampling visits was similar between 2007 and 2008 for both qPCR and MF (Tables 1a and 1b) at both stations. The coefficient of variation (CV) was greater within sampling visits in 2008 versus 2007 for both qPCR and MF with the exception of the qPCR results from Myron/Wilson in 2008 (Tables 1a and 1b.) These higher within-station CV results for 2008 may be attributable to the overall lower amounts of *Enterococcus* measured throughout the study in 2008. The lower SD between sampling visits in Central Avenue

**Tables 1a. and 1b. Comparison of Enterococcus Results and Descriptive Statistics for the Myron/Wilson and Central Avenue Sampling Stations Using Membrane Filtration and qPCR, Summer 2007 and 2008 Studies**.

1a.

| Myron/Wilson, Monmouth County | MF 2007 | MF 2008 | qPCR 2007 | qPCR 2008 |
|---|---|---|---|---|
| Arithmetic Mean of All Sampling Visits | 123 | 170 | 79.5 | 268 |
| Geometric Mean of All Sampling Visits | 64.9 | 27.5 | 37.7 | 17.3 |
| Log 10 SD BETWEEN Sampling Visits | .57 | .68 | .51 | .83 |
| Log10 SD WITHIN Sampling Visits | .23 | .34 | .43 | .41 |
| C.V. WITHIN Sampling Visits | .48 | 1.14 | .84 | .69 |

1b.

| Central Avenue, Ocean County | Enterolert 2007 | MF 2008 | qPCR 2007 | qPCR 2008 |
|---|---|---|---|---|
| Arithmetic Mean of All Sampling Visits | 261 | 12.2 | 549 | 80 |
| Geometric Mean of All Sampling Visits | 150 | 9.4 | 357 | 51.1 |
| Log 10 SD BETWEEN Sampling Visits | .49 | .22 | .49 | .41 |
| Log10 SD WITHIN Sampling Visits | .21 | .22 | .23 | .34 |
| C.V. WITHIN Sampling Visits | .40 | .67 | .31 | .63 |

Note: Enterolert was used as the conventional monitoring parameter for Enterococcus in Ocean County in 2007.

from 2008 may be attributable to the low concentrations of *Enterococcus* detected for both qPCR and MF throughout the eight week sampling period (Tables 1a and 1b). The geometric mean values of *Enterococcus* decreased approximately 50% by both methods at Myron/Wilson in 2008 as compared to 2007 results (Figure 2a). The decrease was more marked at Central Avenue for both qPCR and MF in 2008 (Figure 2b). This was the second year that the geometric mean was higher for MF versus qPCR results. This result is in contrast to other studies (Wade et al 2006; Griffith and Weisburg 2006). Due to cell die off, and the ability for qPCR to measure dead or nonviable cells, it has been found that qPCR results are typically higher when compared to conventional MF. This

Table 2a. Significant differences in *Enterococcus* concentrations at Central Avenue, Ocean County for qPCR and membrane filtration geometric means based on time of day sampled (Morning, 6 hours from the morning sample, and 24 hours from the morning sample).

| ***Date*** | ***Analysis Type*** | ***MID STATION AM*** | ***MID STATION PM*** | ***MID STATION 24 HOURS*** |
|---|---|---|---|---|
| July 10-11, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 16-17, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 23-24, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 30-31, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 6-7, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 11-12, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | **B** | **B** |
| | | | | |
| August 14-15, 2008 | Membrane Filtration | A | **B** | A,B |
| | qPCR | A | A | A |
| | | | | |
| August 20-21, 2008 | Membrane Filtration | A | **B** | A,B |
| | qPCR | A | A | **B** |
| | | | | |
| August 27-28, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |

*Note: Sampling times with the same letter do not have significantly different geometric means (N=4). Significant differences are highlighted in yellow and the letter "B" indicates significantly higher (p=0.05) than the "A" designations.*

Table 2b. Significant differences in *Enterococcus* concentrations at Myron/Wilson (Memorial Park), Monmouth County for qPCR and membrane filtration geometric means based on time of day sampled (Morning, 6 hours from the morning sample, and 24 hours from the morning sample).

| ***Date*** | ***Analysis Type*** | ***MID STATION AM*** | ***MID STATION PM*** | ***MID STATION 24 HOURS*** |
|---|---|---|---|---|
| July 7-8, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 14-15, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 21-22, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 28-29, 2008 | Membrane Filtration | **B** | A | **B** |
| | qPCR | **B** | A | **B** |
| | | | | |
| August 4-5, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 11-12, 2008 | Membrane Filtration | A | **B** | A |
| | qPCR | A | A | A |
| | | | | |
| August 18-19, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 25-26, 2008 | Membrane Filtration | **B** | A | A |
| | qPCR | A | A | A |

*Note: Sampling times with the same letter do not have significantly different geometric means (N=4). Significant differences are highlighted in yellow and the letter "B" indicates significantly higher (p=0.05) than the "A" designations.*

points to the importance of site specific studies to evaluate for these types of occurrences. *Enterococcus* via MF did not exceed the established maximum water quality criterion of 104 CFU/100 mL in seven of the eight sampling events at both Myron/Wilson Bay and Central Avenue. Including all replicates from the entire study (each transect sampling point, afternoon samples, and 24 hour samples), only 2.8% and 9.3% of all samples analyzed by MF exceeded the 104 CFU/100 mL threshold at Central Avenue and Myron Wilson Bay, respectively. Seventy one percent of all samples (125 out of 176) were characterized by *Enterococcus* concentrations of 10 cfu/100 mL or less at Central Avenue and one half of all samples analyzed at Myron/Wilson did not exceed 10 CFU/100 mL (80 out of 160) based on MF data. However, 7.3 and 22.5% of samples from Central Avenue and Myron Wilson Bay respectively were characterized as non detect using qPCR.

## 4.2 Regression Analysis

A scatter plot and regression analysis of qPCR versus MF geometric mean densities of *Enterococcus* from all sampling visits is presented in Figure 3. The overall correlation coefficient (*r*) between qPCR and MF was 0.74. This strong correlation is similar to the value reported in a 2007 study of marine sampling areas by Ferretti et. al. 2008 and by Haugland et. al. 2005 in a qPCR method comparison study of two freshwater bathing beaches. At a study in the Root River and Lake Michigan, the calculated $R^2$ was 0.62 (Lavendar et al 2009). The $R^2$ in this study was 0.55 (Figure 3). The slope of the regression is lower in the 2008 dataset as compared to 2007. This may be due to evaluation of data from only 2 stations in 2008 versus 10 in 2007 and the relatively lower overall *Enterococcus* concentrations estimated in 2008 (Figures 2a and 2b).

## 4.3 Temporal Trends

Figures 4a and 4b represent the geometric means of *Enterococcus* densities at all sampling locations measured over the course of the study. In general, sampling areas with low concentrations of *Enterococcus* as measured by MF also had low levels of *Enterococcus* via qPCR. At Central Avenue, qPCR values were close to an order of magnitude higher through the first two-thirds of the sampling period and this gap closed

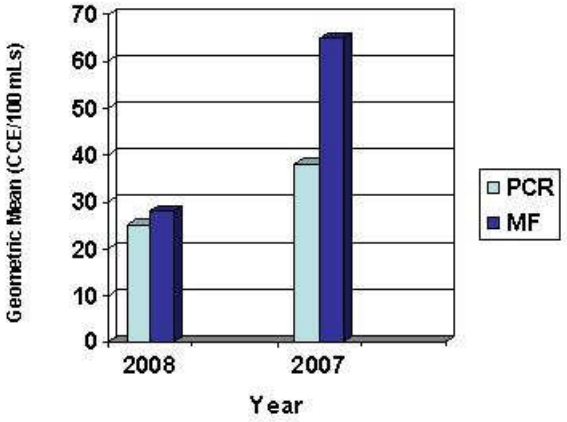


Figure 2a. Myron/Wilson

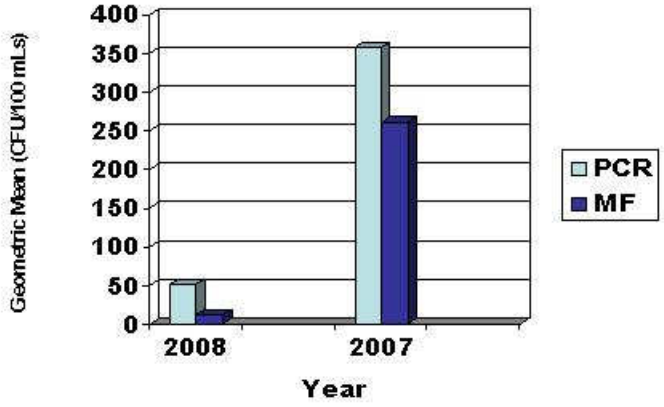


Figure 2b. Central Avenue

Figures 2a. and 2b. Comparison of Average Geometric Means of qPCR and Membrane Filtration Results from 2007 and 2008 study of Enterococcus concentrations at Myron/Wilson Bay (Memorial Park), Monmouth County, NJ and Central Avenue Beach, Ocean County, NJ.

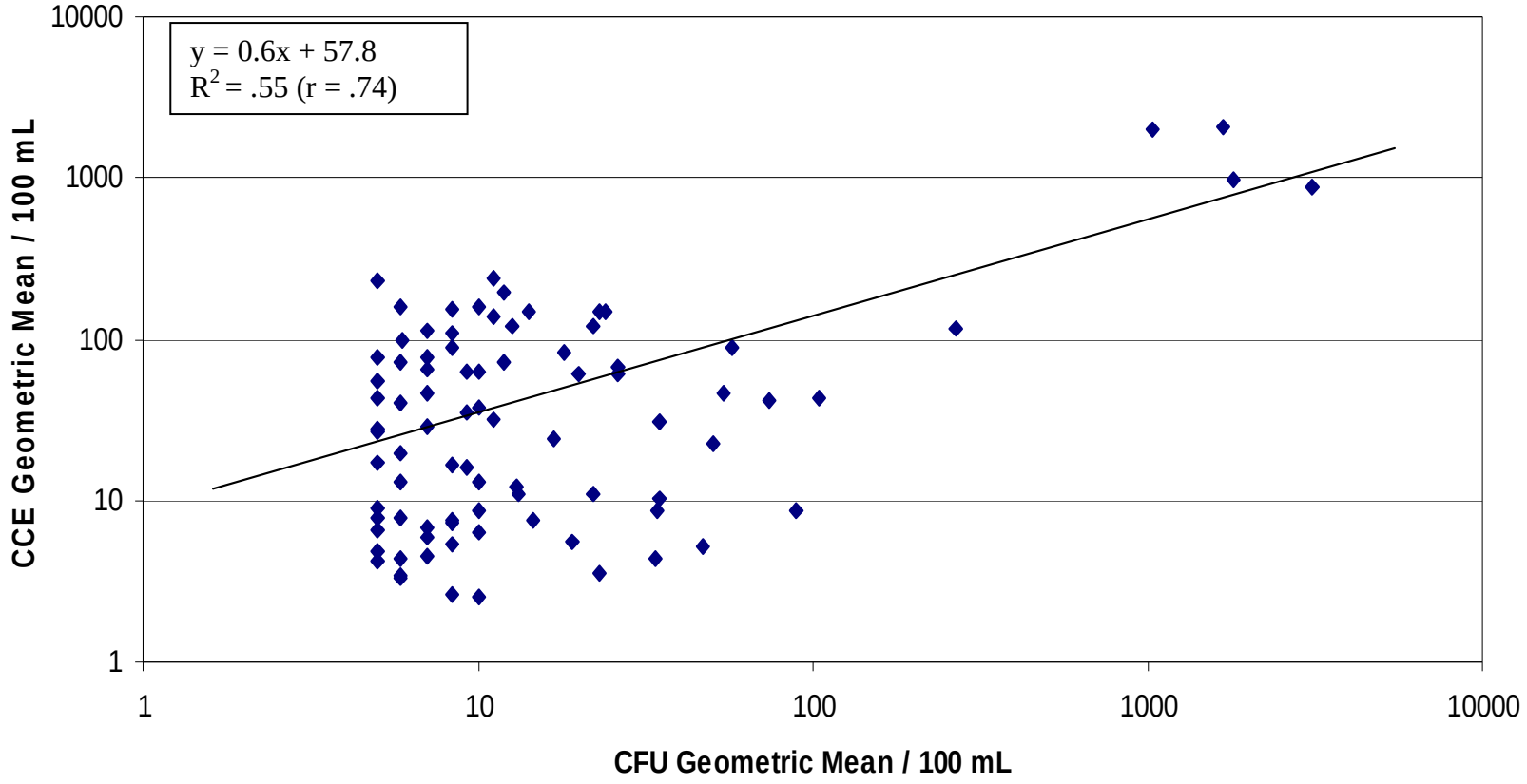


Figure 3. Scatter plot and regression analysis results of geometric mean Enterococcus CFU densities (n=84), determined by Membrane Filtration versus *Enterococcus* CCE, determined by qPCR from samples collected at the mid transect location at Central Avenue (Ocean County) and Myron/Wilson Bay (Memorial Park), Monmouth County between July 7 and August 27, 2008.

during the last 2 weeks of the project (Figure 4a.). This trend was not evident at Myron/Wilson Bay. qPCR and MF results were similar throughout the entire study period (Figure 4b). In general, the relative changes in MF from week to week were reflected in the qPCR values, which generally changed in the same direction and magnitude. qPCR results were almost an order of magnitude higher for the first six weeks of the study and then were comparable over the final two weeks (Figure 4a). There was nothing in physical chemical data that explained this trend.

Four replicates were analyzed in the morning (between 6:00 and 8:00 am); repeated approximately 6 hours later (between 12:00 and 2:00 pm); and then the following morning, biweekly at Central Avenue and Myron/Wilson Bay. Similarity based on multiple comparison testing (p=0.05) are summarized in Tables 2a (Central Ave) and 2b (Myron/Wilson Bay). Results which were similar share the same letter designation (A and/or B). The significant differences are highlighted in yellow. Significant differences based on time and day sampled were detected in both MF and qPCR samples. Five of the eight temporal sampling events with significant differences were MF analysis. Where there were significant differences based on time sampled, samples collected six hours later were significantly lower at Myron/Wilson and significantly higher at Central Avenue. At Myron Wilson, there was only one sampling event where *Enterococcus* concentrations via MF were greater than 104 CFU/mL (July 28-29). The temporal patterns for both analysis followed a similar pattern of high concentrations on the initial sampling event and a significant decline in concentrations in the samples recollected after 6 hours. The 24 hour confirmation counts increased slightly but did not approach the initial concentrations measured 24 hours earlier. Overall, there was not a discernable trend with regard to *Enterococcus* densities between morning and afternoon datasets. Data from the EMPACT Beaches Project (USEPA 2005), using E*nterococcus* by MF, found that indicator levels generally decreased by the afternoon at four of the beaches studied, and there was no discernable trends at one freshwater beach which generally had low levels of *Enterococcus* at all times. Wade, et. al. (2006) found *Enterococcus* concentrations were higher in the afternoon in a study of bathing beach areas at the Great Lakes.

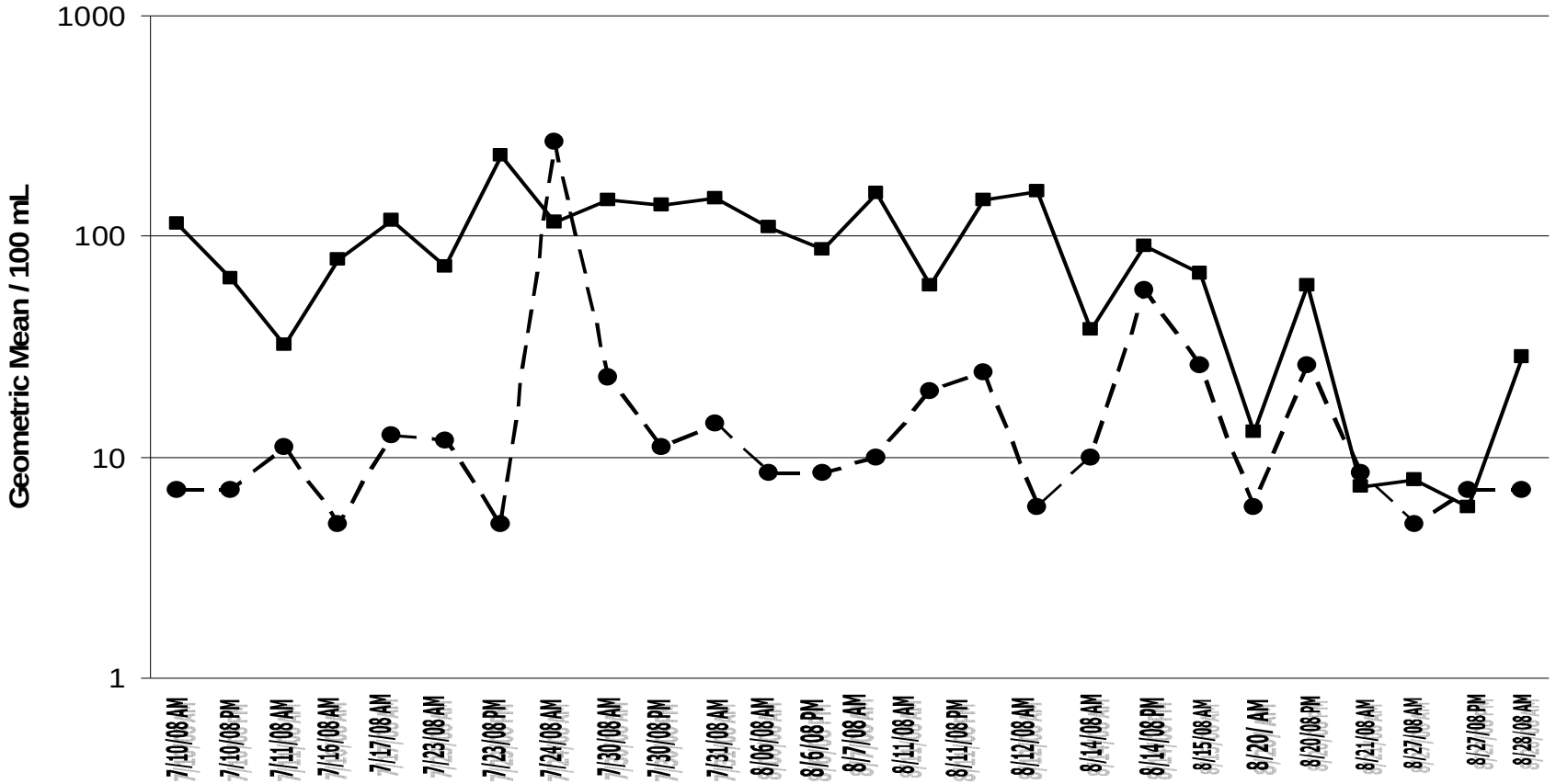


Figure 4a. Geometric means of Enterococcus densities per 100 mL of water samples collected at Central Avenue, Ocean County, NJ. qPCR results are designation with a ( ■ ) on a solid line; and MF results are designation with a ( ●) on a dotted line.

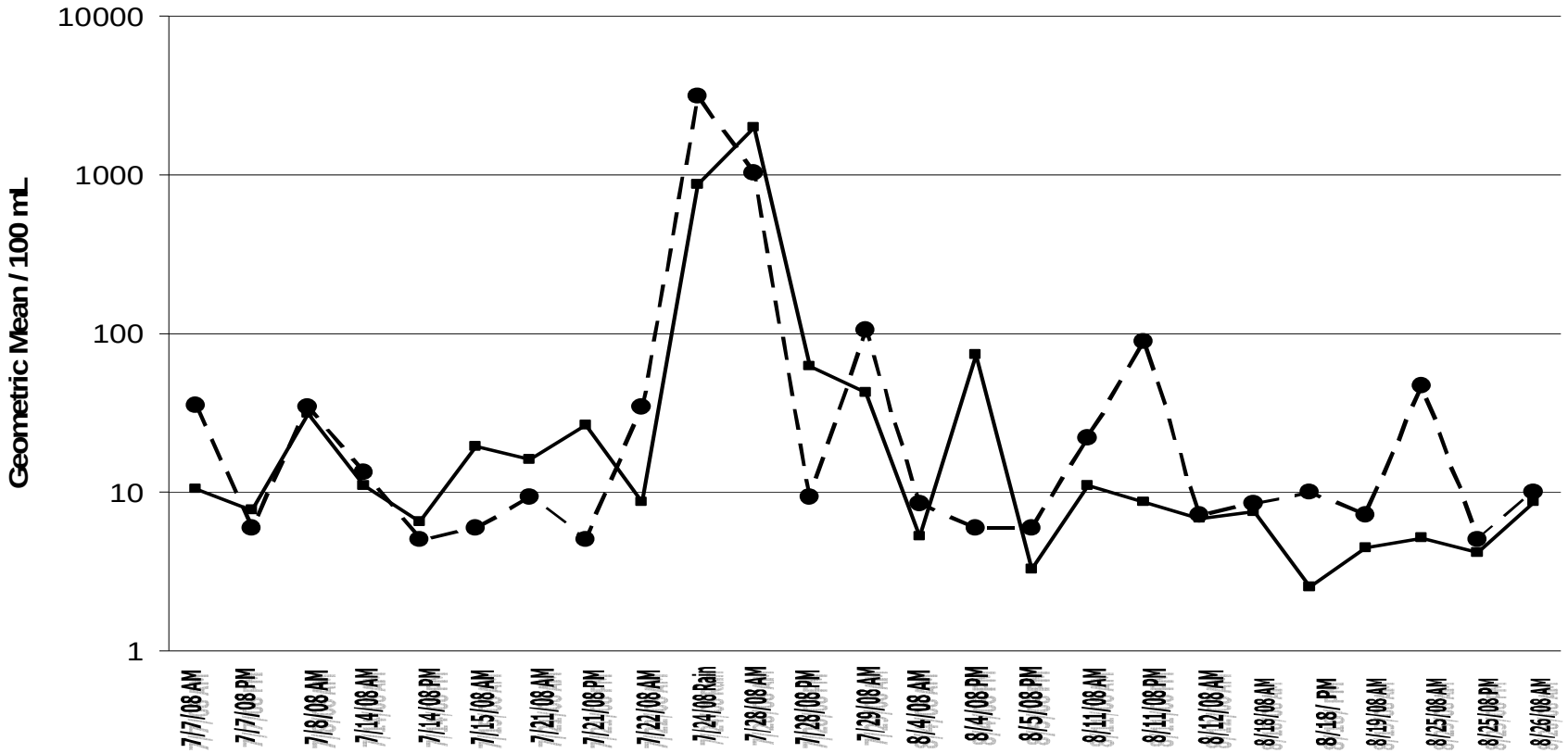


Figure 4b. Geometric means of Enterococcus densities per 100 mL of water samples collected at Myron/Wilson Bay (Memorial Park), Monmouth County, NJ. qPCR results are designation with a ( ■ ) on a solid line; and MF results are designation with a ( ●) on a dotted line.

Telech et al (2009) collected samples at four recreations beach sites in the Great Lakes and found that *Enterococcus* from qPCR did not significantly differ based on different collection times. In a study by Lavendar et al (2009), collection time was not important in modeling Enterococcus using qPCR but time of collection did show significant differences in results from MF tests. Lavendar concluded that commonly used environmental measurements were relatively insensitive in predicting *Enterococcus* estimates via qPCR as compared to their ability to predict results collected via MF. Based on findings from this study, relative levels of bacteria in morning versus afternoon need to be evaluated on a site by site basis. An important study design component of sampling in the morning and 6 hours later was to evaluate changes in *Enterococcus* numbers based on tide height. Relative tide height, as measured by water level above mean low water mark, was seen as a significant determinant of *Enterococcus* density within the swimming areas in a study by Wymer, et. al. 2005. Wave height (and turbidity) was most consistently correlated with *Enterococcus* using qPCR in a study by Telech et al (2009). In our study, the relatively low concentrations of *Enterococcus* throughout most of the sampling event impeded our ability to evaluate temporal effects and relate them to tide height. Overall, temporal variability differences were indiscernible between qPCR and MF methods.

## 4.4 Spatial Trends

Four replicate samples were collected at each station along a transect that spanned approximately 80 meters. The mid station represented the default sampling location and samples were collected 40 M to the left and right of this mid station on 9 occasions at Central Avenue and 8 events at Myron/Wilson (Tables 3a and 3b). Data from the EMPACT Study (Wymer, et. al. 2005) found that bacterial density was similar along a beach front transect as long as the distance from shore was similar. There was only one occurrence in our study where a significant difference in *Enterococcus* concentrations was exhibited along any transect. Overall, spatial differences were not a factor in this study for both MF and qPCR methods. Relatively low levels of *Enterococcus* during the study had an influence on the ability to detect spatial variability that was observed in this study.

Table 3a. Significant differences in *Enterococcus* concentrations at Central Avenue, Ocean County for qPCR and membrane filtration geometric means based on Spatial Variability (40m transect).

| ***Date*** | ***Analysis Type*** | ***LEFT BRACKET*** | ***MID STATION*** | ***RIGHT BRACKET*** |
|---|---|---|---|---|
| July 10, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 16, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | **B** | A,B |
| | | | | |
| July 23, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 30, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 6, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 11, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 14, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 20, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 27, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |

Table 3b. Significant differences in *Enterococcus* concentrations at Myron/Wilson Bay (Memorial Park), Monmouth County for qPCR and membrane filtration geometric means based on Spatial Variability (40m transect).

| Date | Analysis Type | LEFT BRACKET | MID STATION | RIGHT BRACKET |
|---|---|---|---|---|
| July 7, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 14, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 21, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| July 28, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 4, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 11, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 18, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |
| | | | | |
| August 25, 2008 | Membrane Filtration | A | A | A |
| | qPCR | A | A | A |

## 4.5 Physical/Chemical Parameters and *Enterococcus* Concentrations

It has been demonstrated that fecal indicator bacteria densities measured using MF techniques correlate well with certain environmental characteristics such as wind direction, water temperature, and rainfall (Francy and Darner, 2006, Wymer et al 2005). Results from linear regression models in a study by Telech et al (2009) indicate that environmental factors explain more of the variability in *Enterococcus* densities measured by MF than qPCR. Salinity, water temperature, air temperature, cloud cover, wind direction and wind speed, and tidal cycle data were collected in this study (Tables 4 and 5). The data are arranged in a row based on the date sampled. A discussion of some of these variables is provided below.

### *4.5.1 Rainfall*

Rain within 24 hours of sampling produced the strongest relationships between *Enterococcus* using both qPCR and MF in a study by Telech et al (2009). In our study, there were three days in July and two days in August with recorded rainfall amounts over 0.25 inches (http://cdo.ncdc.noaa.gov/qclcd/QCLCD). Bacterial indicator levels may typically be elevated for 24-48 hours after heavy rains as the result of surface runoff or tributary contribution (Genthner et al 2005). Sampling within 24 hours of a rain event coincided with two rain events in July at both Myron/Wilson Bay and Central Avenue. The highest concentrations of *Enterococcus* measured in this study for both MF and qPCR coincided with both of these rain events (July 24 and July 28).

### *4.5.2 Turbidity*

Rainfall and turbidity generally exhibit a positive correlation. In a study by Telech et al (2009), turbidity had a high positive correlation using qPCR. In our study, turbidity was relatively low for all samples collected throughout the study period (Tables 4 and 5). Only on one occasion did a result exceed 10 NTUs (Myron Wilson, July 28, 2008). This date also coincided with the highest *Enterococcus* concentrations measured using both MF and qPCR.

Table 4a. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Station at Central Avenue Beach, Ocean County, New Jersey, July –August 2008.

CENTRAL AVENUE, OCEAN COUNTY, NJ

| Sampling Week # | 7/10/2008 am | 7/10/2008 pm | 7/11/2008 am | 7/16/2008 am | 7/16/2008 pm | 7/17/2008 am | 7/23/2008 am | 7/23/2008 pm | 7/24/2008 am |
|---|---|---|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 114.2 | 65.2 | 31.6 | 77.7 | ND | 119.3 | 73.2 | 231.8 | 117.4 |
| MF Geometric Mean | 7.1 | 7.1 | 11.1 | 5.0 | ND | 12.6 | 11.9 | 5.0 | 265.8 |
| Salinity | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Turbidity | 4.5 | 6.1 | 4.7 | 3.4 | ND | 5.8 | 4.6 | 7.5 | 5.1 |
| Air Temperature | 72 | 82 | 65 | 65 | 85 | 67 | 75 | 80 | 75 |
| Water Temperature | 76 | 80 | 72 | 75 | 75 | 74 | 75 | 75 | 75 |
| Precipitation | None | None | None | None | None | None | None | None | Rain |
| Tidal Cycle | Ebbing | Ebbing | Flooding | Flooding | Ebbing | Ebbing | Ebbing | Flooding | Ebbing |
| Wind Direction | None | None | None | None | None | None | Calm | None | Calm |
| Cloud Cover | Clear | Clear | Clear | Clear | Clear | Clear | Clear | Clear | Heavy |
| Time Collected | 06:30am | 12:30pm | 06:30am | 06:25am | 12:30pm | 06:30am | 06:45am | 12:30pm | 06:30am |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

Table 4b. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Station at Central Avenue Beach, Ocean County, New Jersey, July –August 2008.

CENTRAL AVENUE, OCEAN COUNTY, NJ

| Sampling Week # | 7/30/2008 am | 7/30/2008 pm | 7/31/2008 am | 8/6/2008 am | 8/6/2008 pm | 8/7/2008 am | 8/11/2008 am | 8/11/2008 pm | 8/12/2008 am |
|---|---|---|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 147.3 | 138.9 | 147.8 | 109.4 | 88.4 | 157.9 | 59.8 | 146.5 | 161.2 |
| MF Geometric Mean | 23.4 | 11.1 | 14.1 | 8.4 | 8.4 | 10.0 | 20.0 | 23.8 | 5.9 |
| Salinity | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Turbidity | Inc. | Inc. | Inc. | Inc. | Inc. | Inc. | 4.7 | 6.3 | ND |
| Air Temperature | 70 | 85 | 78 | 70 | 80 | 66 | 68 | 72 | 60 |
| Water Temperature | 70 | 75 | 80 | 60 | 70 | 60 | 65 | 78 | 68 |
| Precipitation | None | None | None | None | None | None | None | None | ND |
| Tidal Cycle | Flooding | Ebbing | | Ebbing | Flooding | Ebbing | Flooding | Ebbing | Flooding |
| Wind Direction | None | None | None | None | None | None | None | None | None |
| Cloud Cover | Cloudy | Clear | Clear | Cloudy | Clear | Clear | Cloudy | Clear | ND |
| Time Collected | 06:30am | 12:30pm | 06:30am | 06:40am | 12:00pm | 07:11am | 06:24am | 12:10pm | 07:00am |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

Table 4c. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Station at Central Avenue Beach, Ocean County, New Jersey, July –August 2008.

CENTRAL AVENUE, OCEAN COUNTY, NJ

| Sampling Week # | 8/14/2008 am | 8/14/2008 pm | 8/15/2008 am | 8/20/2008 am | 8/20/2008 pm | 8/21/2008 am | 8/27/2008 am | 8/27/2008 pm | 8/28/2008 am |
|---|---|---|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 38.1 | 89.6 | 68.3 | 12.9 | 59.9 | 7.3 | 7.9 | 5.9 | 28.6 |
| MF Geometric Mean | 10.0 | 57.0 | 26.3 | 5.9 | 26.3 | 8.4 | 5.0 | 7.1 | 7.1 |
| Salinity | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Turbidity | 4.6 | 9.5 | 4.7 | 5.4 | 8.0 | 5.0 | Inc. | Inc. | 5.0 |
| Air Temperature | 66 | 75 | 70 | 70 | 75 | 60 | 60 | 70 | 60 |
| Water Temperature | 60 | 65 | 60 | 65 | 65 | 60 | 50 | 65 | 55 |
| Precipitation | None | None | None | None | None | None | None | None | None |
| Tidal Cycle | Flooding | Ebbing | Flooding | Ebbing | Flooding | Ebbing | Flooding | Ebbing | Flooding |
| Wind Direction | None | None | None | None | None | None | None | NE | None |
| Cloud Cover | Clear | Clear | Cloudy | Clear | Clear | Clear | Clear | Clear | Clear |
| Time Collected | 07:00am | 12:00pm | 06:47am | 06:45am | 12:00pm | 07:08am | 07:16am | 11:58am | 06:45am |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

Table 5a. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Location at Myron/Wilson Bay (Memorial Park), Monmouth County, NJ, July –August 2008.

MYRON/WILSON BAY (MEMORIAL PARK), MONMOUTH COUNTY

| **Sampling Date** | 7/7/2008am | 7/7/2008pm | 7/8/2008am | 7/14/2008am | 7/14/2008pm | 7/15/2008am | 7/21/2008am | 7/21/2008pm | 7/22/2008am | 7/24/2008am |
|---|---|---|---|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 10.4 | 7.7 | 30.9 | 11.0 | 6.5 | 19.5 | 16.0 | 26.7 | 8.7 | 868.6 |
| MF Geometric Mean | 34.8 | 5.9 | 34.6 | 13.2 | 5.0 | 5.9 | 9.3 | 5.0 | 34.1 | 3095.5 |
| Salinity | 27.6 | ND | 27.3 | 28.7 | ND | 30.6 | 30.4 | ND | 31 | ND |
| Turbidity | 5.23 | ND | 3.69 | 5.18 | ND | 2.46 | 3.78 | ND | 1.42 | ND |
| Air Temperature | 22 | 26 | 25 | 21 | 23.8 | 24.5 | 27 | 32.2 | 23 | 20.5 |
| Water Temperature | 21.1 | 22 | 22.3 | 23.4 | 22.7 | 21.7 | 22.7 | 19.3 | 20.8 | 20 |
| Precipitation | .01” | None | None | None | .05” | None | None | None | None | 1.12 |
| Tidal Cycle | Low | High | Low | High | High | High | Low | High | Low | Low |
| Wind Direction | NE | NE | W | NNE | NW | NW | NW | NE | SSE | SSE |
| Cloud Cover | Cloudy/Fog | Clear | Clear | Cloudy | Cloudy | Sunny | P. Cloudy | Sunny | Cloudy | Rain |
| Time Collected | 0748 | 1320 | 0811 | 0737 | 1333 | 0812 | 0738 | 1340 | 0821 | 0915 |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

Table 5b. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Location at Myron/Wilson Bay (Memorial Park), Monmouth County, NJ, July –August 2008.

MYRON/WILSON BAY (MEMORIAL PARK), MONMOUTH COUNTY NJ

| **Sampling Date** | 7/28/2008am | 7/28/2008pm | 7/29/2008am | 8/4/2008am | 8/4/2008pm | 8/5/2008am | 8/11/2008am | 8/11/2008pm | 8/12/2008am |
|---|---|---|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 2003.5 | 61.6 | 42.8 | 5.3 | 7.3 | 3.3 | 11.0 | 8.7 | 6.9 |
| MF Geometric Mean | 1031.9 | 9.3 | 104.3 | 8.4 | 5.9 | 5.9 | 22.1 | 88.6 | 7.1 |
| Salinity | 16 | ND | 41.98 | 43.17 | ND | 24.9 | 28 | ND | 24.3 |
| Turbidity | 13.6 | ND | 4.56 | 3.06 | ND | 2.06 | 6.19 | ND | 3.61 |
| Air Temperature | 24 | 27 | 24 | 23 | 28.5 | 21 | 21.2 | 21.1 | 20 |
| Water Temperature | 21 | 21.1 | 20.5 | 22.6 | 23.7 | 22.5 | 22.1 | 24.3 | 20.1 |
| Precipitation | ND | 0.86 | None | None | None | None | .01 | 0.26 | None |
| Tidal Cycle | Low | High | High | Mid | High | Low | High | Low | High |
| Wind Direction | S | S | NW | NW | NW | NNW | NW | NNE | NE |
| Cloud Cover | ND | Sunny | Sunny | Sunny | Sunny | Sunny | Sunny | Cloudy | Sunny |
| Time Collected | 0753 | 1335 | 0832 | 0744 | 1332 | 0846 | 0742 | 1350 | 0816 |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

Table 5c. Summary of Chemical and Physical Parameters For Each Sampling Visit at the Mid Transect Sampling Location at Myron/Wilson Bay (Memorial Park), Monmouth County, NJ, July –August 2008.

MYRON/WILSON BAY (MEMORIAL PARK), MONMOUTH COUNTY, NJ

| **Sampling Date** | 8/18/2008 am | 8/18/2008 pm | 8/19/2008 am | 8/25/2008 am | 8/25/2008 pm | 8/26/2008 am |
|---|---|---|---|---|---|---|
| qPCR Geometric Mean | 7.5 | 2.5 | 6.3 | 5.1 | 4.2 | 8.7 |
| MF Geometric Mean | 8.4 | 10.0 | 7.1 | 46.8 | 5.0 | 10.0 |
| Salinity | 28.9 | ND | 29.8 | 23.3 | ND | 26.9 |
| Turbidity | 4.64 | ND | 5.68 | 8.07 | ND | 9.98 |
| Air Temperature | 22.2 | 29.5 | 23.5 | 23.5 | 25.4 | 19 |
| Water Temperature | 23.2 | 26.1 | 24.3 | ND | 24.8 | 21.1 |
| Precipitation | None | None | None | None | None | None |
| Tidal Cycle | Low | High | High | High | Mid | Mid |
| Wind Direction | WNW | SSW | W | W | SSE | W |
| Cloud Cover | Sunny | Sunny | Sunny | Cloudy | Cloudy | Sunny |
| Time Collected | 0738 | 1336 | 0809 | 0738 | 1330 | 0718 |

Table Notes:
Air and Water Temperature = °Fahrenheit; Turbidity = NTUs; ND = No Data; S = South, W= West, N= North, E= East; qPCR Units= CCE/100 mL; MF Units= CFU/100 mL

### *4.5.3 Cloud Cover*

It has been suggested that pathogens measured using qPCR technology may be more persistent in the environment than those measured using MF (Sagarin, 2009); e.g. less likely to decline due to sunlight (Noble 2006); or chlorine disinfection (He and Juiang, 2005). In a study by Lavendar et al (2009), there appeared to be a relative insensitivity of qPCR measurements to sunlight deactivation compared to culture based measurements. We used cloud cover to predict sunlight in our study. Again, consistent levels of *Enterococcus* below the risk threshold values affected our ability to associate percent cloud cover (sun light) with qPCR and MF test results.

### *4.5.4 Tide Cycle/Tide Height and Wind Direction*

In our study, we were especially interested in the effects of tide height on *Enterococcus* concentrations among the 6 hour and 24 hour samples as compared to the original sample collected in the morning of each weeks sampling event. Tide height has been shown to have a positive relationship with certain pathogens (Telech et al 2009). Pathogen levels may also be elevated when waves are high due to the re-suspension of microorganisms associated with bottom sediments. Wave height is affected by wind speed and direction along the swimming zone. Bacterial concentrations may be affected by both on- and off-shore winds due to the potential to stir up surf sediments that could re-suspend pathogens. The effects on-shore versus off-shore winds may be more of a beach specific phenomenon. The lower than expected *Enterococcus* concentrations at both study areas prevented determination of meaningful relationships between the test methods and cycle/tide height, wind direction, cloud cover, water and air temperature on bacterial concentrations.

## 5.0 CONCLUSIONS

qPCR was found to provide accurate and sensitive measurements of *Enterococcus* sp. concentrations and was performed in less than 4 hours per batch. This study supports the continued evaluation of qPCR as a potentially effective monitoring tool for bathing beach management.

In summary:

• Estimates of *Enterococcus* densities by the qPCR method and measurements by conventional MF methods exhibited similar levels of between visit variability and within visit variability.

• A significant positive correlation was observed between the qPCR and MF results over all sampling areas, similar to the 2007 study, supporting the original finding that the qPCR method has the potential to be used as a tool for beach management.

• There were no differences in spatial variability between qPCR and MF, although the *Enterococcus* levels were relatively low for both qPCR and MF throughout the eight week study period. *Enterococcus* concentrations of 10 CFU/100ml or less were measured in 53% of the Myron Wilson and 61% of the Central Avenue samples analyzed using MF. *Enterococcus* via qPCR was detected at a greater percentage than MF results, but qPCR results were overall relatively low throughout the study period.

• There were some differences in temporal variability, but there were no discernable trends for a particular time of day or endpoint. In general, qPCR and MF results changed in the same direction as *Enterococcus* levels increased or decreased throughout the study period. Beach site specific studies should be further evaluated to determine the temporal variability characteristics.

### *5.1.1 Future/Ongoing Needs*

There is a need is to collect epidemiological data in conjunction with qPCR data to help formulate appropriate risk values. Epidemiological studies are being performed by USEPA as part of the NEEAR program using qPCR data and the Method 1600 MF procedure. The objective of the NEEAR program is to evaluate the water quality at one or two beaches per year and ultimately obtain a new set of health and water quality. Also, there is a need to evaluate qPCR protocols using different real time PCR instruments that are currently available. Different instruments may give slightly different results due to different optical and thermal cycling capabilities. There is a need to

determine if these units are able to provide similar raw cycle threshold measurements as well as quantitative estimates of target sequences after calibration of the CT measurements. The Office of Research and Development of USEPA has completed a study and USEPA Region 2 has initiated a similar study comparing qPCR results using up to three of the more common thermal cyclers (Cepheid, Roche, ABI). Evaluation of qPCR variability, epidemiological data, and qPCR instrumentation are all important components needed to help establish a human health criterion for *Enterococcus* at marine bathing beaches using qPCR.

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Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

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